Reference : DSHB3067

Technical data sheet

Product :
Microinstant ® *Listeria* Selective Agar Base according to Ottaviani and Agosti

Specification

Medium for the isolation of *Listeria spp.* and the presumptive identification of *L. monocytogenes* acc. to ISO 11290-1 and 11290-2 standard.

Formula * in g/L

Meat peptone	18.000
Casein peptone	6.000
Yeast extract	10.000
Sodium pyruvate	2.000
Dextrose	2.000
Magnesium glycerophosphate	1.000
Magnesium sulphate	0.500
Sodium chloride	5.000
Lithium chloride	10.000
Disodium phosphate anhydrous	2.500
5-bromo-4-chloro-3-indolyl-β-D-glucopyranoside	0.050
Agar	13.000

Final pH 7.2 ± 0.2 at 25 ºC

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 35 g of powder in 476 ml of purified water and bring to the boil with constant stirring. Sterilise by autoclaving at 121ºC for 15 minutes. Cool to 45-50ºC and add 1 botlle of Listeria enrichment supplement Ottaviani & Agosti (Art. DSHB3072) and 1 vial of Listeria selective supplement Ottaviani & Agosti (Art. DSHB3071). Homogenize by mixing and distribute in Petri dishes. The solidified medium appears turbid.

Description

Completed with all its supplements the Agar Listeria O&A is a selective and differential medium for the detection of Listeria species and the presumptive identification of *Listeria monocytogenes.*
The selectivity is achieved by the high concentration of lithium chloride and the mixture of antimicrobics. The differential activity is due to the chromogenic substrate to detect the β-glucosidase, enzyme that is present in all Listeria species.
The specific identification is obtained by the L-α-phosphatidylinositol, that acts as substratre for a phospholipase C that is present only in *Listeria monocytogenes* and some strains of *Listeria ivanovii.*
The combination of both substrates allows the differentiation *L. monocytogene*s that produces colonies blue-green in colour but surrounded by an opaque zone fron the other Listeria species that growth with blue-green colonies without any halo. This differentiation is evident after incubate the plates for 24±2 hours at 37ºC.
Sometimes, especially with highly contaminated samples it is possible that can growth some colonies, white in colour, that are not Listeria. In this case it is recommended an enrichment step previous to the plate inoculation.
Observations: Most Listeria ivanovii also produce an opaque halo around the colonies after 48 h of incubation. This presumptive evidence must be confirmed by performing the biochemical or serological identification tests (Ramnosa / Xylose sugar fermentation, hemolysis tests, CAMP test, etc.) or any test confirming the species without hesitation.

Remarks:
Listeria enrichment supplement Ottaviani & Agosti (Art. DSHB3072):
1 vial sufficient amount for 500ml complete medium

L-α-phosphatidylinositol	1.0 g
Steril distilled water	24.0 ml

Listeria selective supplement Ottaviani & Agosti (Art. DSHB3071):
1 vial sufficient amount for 500ml complete medium

Nalidixic acid	10.0 mg
Ceftazidime	10.0 mg
Cycloheximide	25.0 mg
Polymyxin B sulphate	38350 ui

Technique

There are many standardised methodologies (ISO, FDA-BAM, AOAC, AFNOR, etc.). The technician must follow the protocol validated in his laboratory.

Quality control

Incubation temperature: 37 ± 1 °C **Incubation time:** 44±4 h

Inoculum: Practical range 100 ± 20 CFU. min. 50 CFU (productivity)/ 10^4-10^6 CFU (selectivity)/ ≥ 10^3 CFU (specificity), according to ISO 11133:2014/Amd 1:2018 & Adm 2:2020

Microorganism	Growth	Remarks
Escherichia coli ATCC® 25922	Inhibited	-
Listeria monocytogenes ATCC® 13932	Productivity > 0.50	green-blue colonies surrounded by opaque halo
Listeria monocytogenes ATCC® 35152	Productivity > 0.50	green-blue colonies surrounded by opaque halo
Listeria inocua ATCC® 33090	Good (specificity)	green-blue colonies without opaque halo
Enterococcus faecalis ATCC® 29212	Inhibited	-

References

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·Bannerman, E.S. & J. Bille (1988) A new selective medium for isolating Listeria from heavily contaminated material. Appl.m Environm. Microbiol. 54:1:165-167.

·Greenwood, M., C. Willis, P. Dosweell, G. Allen & K. Pathak (2005) Evaluation of chromogenic media for the detection of Listeria species in food.

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. ISO 11133:2014/ Adm 1:2018/ Adm 2:2020/ Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.

· ISO 11290-1:2017 Standard. Microbiology of the food chain. Horizontal method for the detection and enumeration of Listeria monocytogenes and for Listeria spp.- Part 1: Detection Method

· ISO 11290-2:2017 Standard. Microbiology of the food chain. Horizontal method for the detection and enumeration of Listeria monocytogenes and for Listeria spp.- Part 2: Enumeration Method

·Jantzen, M.M., J. Navas, M. de Paz, B. Rodriguez, W.P. da Silva & M. Nuñez (2006) Evaluation of ALOA plating medium for its suitability to recover high pressure-injured Listeria monocytogenes from ground chicken meat. Letters Appl. Microbiol 43:313-317

·Manafi, M. W. Kneifel & S. Bascomb (1991) Fluorogenic and chromogenic substrates used in bacterial diagnostics. Microbiol Rev. 55:3:335-348

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·Watkins, J. & K.P. Sleath (1981) Isolation and enumeration of Listeria monocytogenes from sewage, sewage sludge and river water. J. Appl. Bacteriol. 50:1-9

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 ºC to 30 ºC).